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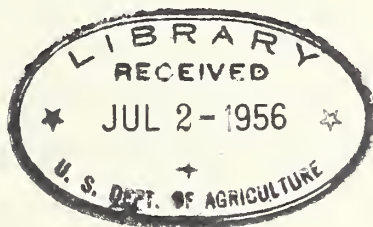
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RS - SS  
RS - SE  
GENETICS  
Racial variation

September 12, 1952

WORKING PLAN FOR COOPERATIVE STUDY OF  
GEOGRAPHIC SOURCES OF SOUTHERN PINE SEED

By the Committee on Southern Forest Tree Improvement,  
Subcommittee on Geographic Source of Seed.



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Previous publications and reports in cooperation with the Committee on Southern Forest Tree Improvement are:

1. Report of the first southern conference on forest tree improvement. Atlanta, Georgia, January 9-10, 1951. Mimeographed. U. S. Forest Service, Atlanta, Georgia.
2. Proposal for a cooperative study of geographic sources of southern pine seed. Subcommittee on Geographic Source of Seed, Philip C. Wakeley, Chairman. Mimeographed. Southern Forest Experiment Station, New Orleans, Louisiana. October 1, 1951.
3. Standardized working plan for local tests of seed source. Subcommittee on Geographic Source of Seed, Philip C. Wakeley, Chairman. Mimeographed. Southern Forest Experiment Station, New Orleans, Louisiana. October 25, 1951.
4. Hereditary variation as the basis for selecting superior forest trees. Subcommittee on Tree Selection and Breeding, Keith W. Dorman, Chairman. Southeastern Forest Experiment Station, Station Paper No. 15. March, 1952.
5. Directory of forest genetic activities in the South. Subcommittee on Tree Selection and Breeding, Keith W. Dorman, Chairman. Southeastern Forest Experiment Station, Station Paper No. 17. July, 1952.
6. Working plan for cooperative study of geographic source of southern pine seed. Subcommittee on Geographic Source of Seed, Philip C. Wakeley, Chairman. Processed. Southern Forest Experiment Station, New Orleans, Louisiana. September 12, 1952.

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INTRODUCTION

This is the working plan for a cooperative, region-wide study of geographic sources of the seed of longleaf pine (Pinus palustris, Miller), slash pine (Pinus caribaea Morelet of the 1944 Check List of the United States Forest Service, as it occurs in the continental United States), loblolly pine (Pinus taeda, L.), and shortleaf pine (Pinus echinata, Miller). The study excludes the botanical variety of Pinus caribaea known as "South Florida slash pine," Pinus caribaea from Cuba and other points outside the United States, all minor southern pines such as Virginia scrub, pitch, pond, spruce, and sand pines, and all hybrids.

The origin of the Subcommittee preparing this plan and negotiating for the help to carry it out, and the background of the study itself (including a list of pertinent references) have been described in a mimeographed "Proposal for a Cooperative Study of Geographic Sources of Southern Pine Seed," dated October 1, 1951. Copies of this Proposal have been sent to most or all cooperators already approached; additional copies are or will be made available at the Southeastern and Southern Forest Experiment Stations as needed.

Main Plan and Later Supplements

The present plan describes the purpose and specifies the general design and general execution of the cooperative, region-wide study. It will be followed by several supplements. Supplement A will detail the sources from which seed for the study was obtained in 1951. Supplement B will detail the cooperators producing nursery stock in 1952, and the seed lots assigned to each. Supplement C will detail the cooperators undertaking the establishment of test plantations with stock produced in 1952, and the stock to be planted by each; completion of this supplement will require considerable correspondence, together with a September 1952 inventory of stock available

at each cooperating nursery. Supplement D will cover certain phases of the study postponed until 1952-1953 or later because of lack of seed in 1951 or shortages of nursery stock in 1952. Later supplements may amend present specifications for plantation reexaminations and will give specifications for thinning the test plantations.

It is the intention of the Subcommittee to supply every cooperator in the study, regardless of the nature and extent of his cooperation, with a copy of this working plan and, as they appear, with a complete file of the supplements.

#### Identity of Study; Correspondence About It

Two things require explanation to clear up certain misunderstandings which have already arisen, and to prevent their recurrence.

One is the identity of the study. The present study is the first cooperative study of geographic sources of forest tree seed sponsored by the Committee on Southern Forest Tree Improvement. Its cooperative nature is emphasized because its success hinges upon uniform, coordinated action by many agencies and individuals in some 15 different States. It is, however, distinct from and independent of other cooperative studies of geographic sources of seed, such as the one of longleaf, slash, loblolly, and shortleaf pines attempted by the Southern Forest Experiment Station with seed from the 1935 crop, and the more promising cooperative study of loblolly pine seed sources initiated by the Tennessee Valley Authority in 1949-1950. It is also distinct from strictly local tests of geographic sources of seed by individual agencies, such as the study of four sources of loblolly pine seed established by the Southern Forest Experiment Station at Bogalusa, Louisiana, with seed from the 1925 crop, or studies arising from the distribution of the mimeographed "Standardized Working Plan for Local Tests of Geographic Sources of Seed" dated October 25, 1951.

The second thing which requires explanation is correspondence concerning the present study. As the Southern Forest Experiment Station is, in effect, the "chairman organization" of the Subcommittee initiating the study, and as cooperators are situated partly in the territory of that Station and partly in the territories of three others, advantage naturally has been taken of the facilities of the four Experiment Stations to find and negotiate with the cooperators and to coordinate their work. This, however, has necessitated correspondence through prescribed Station channels, instead of directly between the chairman of the Subcommittee and the cooperators. In particular, the chairman may approach cooperators in the territories of the Central States, Northeastern, and Southeastern Stations only through the Directors of those Stations. The resulting letters bear a great variety of signatures--often those of Division Chiefs or Acting Directors--but this variety should cause cooperators no concern so long as the letters clearly



refer to the present cooperative, region-wide study. Unless some other action is specifically suggested in the correspondence itself, it will be best to address replies to the Director of the Station from which the correspondence came.

#### Expense and Work Involved in Cooperation

As stated in the mimeographed Proposal of October 1, 1951, cooperation in the present study must be entirely at the cooperator's expense except for the cost of incidental correspondence, conferences, seed shipments, and the like. Both the Committee on Southern Forest Tree Improvement and its Subcommittee on Geographic Source of Seed lack independent finances, and can act only as initiating, coordinating, and advising agencies. Cooperators should not undertake to participate unless prepared to finance their undertakings.

Cooperation may consist of any or all of the following: (a) contributing seed from known and described sources; (b) producing and shipping correctly labeled nursery stock; and (c) outplanting stock according to exact specifications, recording details of establishment, protecting the plantations, and periodically making specified remeasurements of part of the planted trees.

Rather definite top limits have been set to the quantities of seed, stock-production, or planting any one cooperator will be asked to undertake in connection with any one geographic source of any one species. These limits are described at appropriate points in the following pages. The total undertaking asked of each individual cooperator will depend on the total number of sources and species with which he is asked to help, and will be stated clearly in the course of negotiations with the cooperator.

#### Cooperators' Rights and Responsibilities

Contributed seed in excess of that needed for nursery sowing, for germination tests to guide nursery sowing, and for small special studies to round out the main study, will be returned to the contributing cooperators.

Distribution of all nursery stock produced in connection with the study will be in accordance with this working plan, its supplements, and any necessary special instructions by the Subcommittee in charge. The present plan specifies sowing for slightly more than twice as much stock of each lot at each nursery as will actually be needed. Scantiness of seed lots from some sources, however, plus even the ordinary hazards of nursery stock production, may result in shortages rather than surpluses of some lots of stock at some nurseries. In such cases, the Subcommittee will draw on nurseries with excesses of the same lots to make good the deficiencies.

All surpluses of nursery stock left after meeting such needs will, regardless of seed source, remain the property of the agencies producing the stock, to be disposed of as they see fit. It is suggested, however, that these surpluses be used, alone or in combination with stock representing still other seed sources, in independent, local tests of geographic sources of seed. Copies of the October 25, 1951, "Standardized Working Plan for Local Tests of Geographic Sources of Seed" may be obtained from the Southern Forest Experiment Station, New Orleans, Louisiana, or the Southeastern Forest Experiment Station, Asheville, North Carolina, as a guide in establishing such independent tests.

With the possible exception of a few specimens for analysis of wood quality, all intermediate and final products from any plantation established as part of the cooperative study will belong to the cooperator doing the outplanting, or to his successor in ownership. Intermediate and final cuttings will, however, be made as and when specified in this working plan and its supplements, to insure comparability of results in all localities in which outplanting is done.

Individual cooperators will be free to publish results of any measurements and analyses they may wish to make of seedlings they produce or plantations they establish as part of the cooperative study. The Committee on Southern Forest Tree Improvement, through its Subcommittee on Geographic Sources of Seed, will review such publications in manuscript if the authors wish, to insure accuracy and consistency of statement and emphasis. The Committee may be addressed in care of either the Southern or Southeastern Forest Experiment Stations.

Publication of results obtained with one or more species at several or all nurseries or planting localities is the responsibility of and is reserved for the Subcommittee on Geographic Source of Seed, or for subject-matter specialists, such as geneticists or forest pathologists, to whom the Subcommittee may formally delegate the task, and will be reviewed in manuscript by the Committee on Southern Forest Tree Improvement.

#### PURPOSE OF THE STUDY

The General object of this and other studies of geographic sources of southern pine seed is to map, for each species studied, the zones or territories within which it pays to move seed freely from collecting ground to planting site, but across the boundaries of which seed should be moved cautiously, if at all.

Such zones or territories would represent the distribution of geographic races within the range of the species in question. Theoretically, they could be mapped for any one species by collecting



seed at, say, 50-mile intervals throughout the species range, and, at every point of collection, establishing test plantations with stock grown from seed collected at that point and at every other collecting point in the range.

Practically, such an approach requires more seed, nursery facilities, planting facilities, and research manpower than are available for the present study.

The present study will therefore use seed collections and test plantations of more moderate number and size to learn the influences (temperature, rainfall, and the like) which have given rise to geographic races of southern pines, or with which such races are associated. This information will then be combined with available data on temperatures, etc., to prepare tentative maps of seed collecting zones.

Under this alternative approach, the specific objects of the present cooperative study are: (a) to learn, for each of the four principal species of southern pines, which of several conflicting hypotheses best explains the probable origin and present distribution of such geographic races as may exist; and (b) to rough out, from the substantiated hypotheses and from other pertinent data (as maps of temperature, rainfall, or geological formations) a first approximation to collecting zones for each species. These first approximations should be considerably more useful than present guides to choice of southern pine seed sources. They can be verified, extended, and refined in the light of experience and of later studies specifically designed for the purpose.

In very general terms, the conflicting hypotheses to be tested as referred to in (a) above are:

1. That distinct geographic races of southern pines are associated primarily with temperature zones, and are independent, or nearly so, of differences in rainfall, soil, and the like within any one temperature zone.
2. That distinct geographic races of southern pines are associated with differences in rainfall, soil, and the like, even within the same temperature zone, or independently of temperatures.
3. That distinct geographic races of southern pines bear less relationship to present temperature and rainfall zones, major soil types, and the like than to origin upon or migration from different ancient land masses separated by the seas which formerly covered the present southern coastal plains.
4. That distinct geographic races of southern pines do not exist.

These hypotheses are discussed more fully in the appendix, pages 34 and 35.

## EXPERIMENTAL DESIGN

From 1 to 3 series of 6 to 9 seed lots apiece will be used to test the relative soundness of the various hypotheses concerning the geographic races of the four southern pines included in this study. The number of series per species and of seed lots per series will be determined principally by the extent of the species range and by the variation in temperature, rainfall, major soil types, and geological formations within the range, but in part, necessarily, by the availability of seed and by the location, interest, and facilities of cooperators.

Each seed lot included in any one series will represent a different geographic source, ordinarily at least 100 miles from any other source represented in the same series.

In the species in which seed lots are grouped into 2 or 3 series, certain sources represented in two different series will be identical. The comparable seed lots assigned to different series to represent these identical sources will serve as connecting links between series and will correspondingly broaden and strengthen the conclusions from the study.

Table 1 shows the seed sources that will be studied, the grouping into series of the seed lots representing these sources, the extent to which each series tests the various hypotheses, the sources common to different series, and the inclusion of certain alternative sources as safeguards against lots of seed below standard in quantity or quality.

In testing the various hypotheses, each series within a species will be treated as a unit, independently of any other series.

In the vicinity of each seed source in a series (that is, within 50 and preferably within 5 to 10 miles of the stand or group of stands from which the lot of seed representing that source was collected) there will be established a test plantation containing 4 replications of stock from each and every lot of seed in the series, in balanced, randomized blocks, as illustrated in figure 1. (Further details concerning size and arrangement of plots within blocks are discussed later, and are shown in figure 2.) By this means the survival, growth, cold-resistance, and disease-resistance of stock from every seed lot in the series can be observed both at its own point of origin and at the point of origin of every other lot in the series. (As an extreme example, in Shortleaf Series 2, the behavior of shortleaf from Texas seed can be observed in both Texas and Pennsylvania, and that of shortleaf from Pennsylvania seed can be observed in both Pennsylvania and Texas.) The 4 replications in balanced, randomized block design will permit calculation of the significance of any differences in survival, growth, etc., observed in any one of the 6 or more test plantations of the series.

Table 1.--Grouping of geographic sources of southern pine seed into series to test the relative soundness of four conflicting hypotheses concerning geographic races

State	Source		Approx- average annual temper- ature	(1) Included also in; or (2) Alter- nate source to:-
	County or district	Geological or physiographic province		

°F

LONGLEAF PINE SERIES 1 - "Botanical Origin and Migration Series"  
(Emphasis on hypothesis 3; bears also on 2 and a little on 1 and 4)

Ga.	Treutlen County	Coastal plain	67	(1) Longlf. Series 3
Ala.	Baldwin County	Coastal plain	67	(1) Longlf. Series 2
La.	Washington Parish	Coastal plain	67	---
La.	Rapides Parish	Coastal plain	67	(1) Longlf. Series 3
Texas	Polk, Tyler, and Hardin Counties	Coastal plain	67	--
Ala.	Cleburn County	Appalachian mountain	62	--

LONGLEAF PINE SERIES 2 - "Soils and Temperature Series"  
(Emphasis primarily on hypotheses 2 and 1; secondarily on 4)

N. C.	Bladen County	Coastal plain	62	---
N. C.	Richmond and Moore Counties	Sandhills	62	--
S. C.	Florence and Horry Counties	Coastal plain	64	(1) Longlf. Series 3
S. C.	Chesterfield County	Sandhills	64	---
Ala.	Baldwin County	Coastal plain	67	(1) Longlf. Series 1
Fla.	Okaloosa County	Sandhills	67	--

LONGLEAF PINE SERIES 3 - "Main Temperature Series"  
(Emphasis primarily on hypothesis 1; secondarily on 2, 3,  
and 4; planting deferred for lack of 1951 seed.)

Va.	(Southeastern)	Coastal plain	60	--
S. C.	Florence and Horry Counties	Coastal plain	64	(1) Longlf. Series 2
Ga.	Treutlen County	Coastal plain	67	(1) Longlf. Series 1
Miss.	Harrison County	Coastal plain	67	---
La.	Rapides Parish	Coastal plain	67	(1) Longlf. Series 1
Fla.	(Extreme southern)	Coastal plain	72	--



Table 1.--Grouping of geographic sources of southern pine seed into series to test the relative soundness of four conflicting hypotheses concerning geographic races (continued)

Source			Approx- average annual temper- ature	(1) <u>Included also in; or (2) <u>Alter- nate source to:-</u></u>
State	County or district	Geological or physiographic province		

°F

SLASH PINE SERIES 1 - "Temperature Series"  
(Emphasis primarily on hypotheses 4 and 1)

S. C.	Collettin County	Coastal plain	66	--
Ala.	Monroe County	Coastal plain	66-67	--
Miss.	Harrison County	Coastal plain	67	--
La.	St. Tammany Parish	Coastal plain	67	--
Fla.	Baker County	Coastal plain	68	--
Fla.	Polk County	Coastal plain	72-73	--



Table 1.--Grouping of geographic sources of southern pine seed into series to test the relative soundness of four conflicting hypotheses concerning geographic races (continued)

State	Source		Approx. average annual temper- ature	(1) <u>Included also</u> <u>in</u> ; or (2) <u>Alter-</u> <u>nate source to:-</u>
	County or district	Geological or physiographic province		

OF

LOBLOLLY PINE SERIES 1 - "Temperature Series"

(Emphasis primarily on hypothesis 1; to less extent on 2, 3, and 4)

Md.	Somerset County	Coastal plain	57	--
N. C.	Onslow County	Coastal plain	62	(1) Lob. Series 2
N. C.	Pamlico County	Coastal plain	62	--
Ala.	Cullman County	Pennsylvanian		
		Series	62	--
Ala.	Jefferson County	Pennsylvanian		
		Series	62	(2) Cullman Co., Ala.
Ark.	Clark County	Mississippi		
		Embayment	62-63	(1) Lob. Series 2
Ga.	Wilcox and Crisp			
	Counties	Coastal plain	67	--
La.	Livingston			
	Parish	Coastal plain	67	(1) Lob. Series 2
Texas	Angelina County	Coastal plain	67	--

LOBLOLLY PINE SERIES 2 - "Botanical Origin and Migration Series"

(Emphasis primarily on hypothesis 3; secondarily on 2; cross-check on 1; incidentally on 4)

N. C.	Onslow County	Coastal plain	62	(1) Lob. Series 1
S. C.	Newberry County	Piedmont	62	--
Ga.	Clarke County	Piedmont	62	--
Ga.	Spalding County	Piedmont	62-63	(2) Clarke County, Ga.
Ala.	Clay County	Algonkian or		
		later Cam-		
		brian Series	62	--
Miss.	Prentiss County	Old peneplain	62	--
Tenn.	Hardeman County	Coastal plain	62	--
Ark.	Clark County	Mississippi		
		Embayment	62-63	(1) Lob. Series 1
La.	Livingston			
	Parish	Coastal plain	67	(1) Lob. Series 1

Table 1.--Grouping of geographic sources of southern pine seed into series to test the relative soundness of four conflicting hypotheses concerning geographic races (continued)

State	Source		Approx. average annual temper- ature	(1) Included also in; or (2) Alter- nate source to:-
	County or district	Geological or physiographic province		

OF

SHORTLEAF PINE SERIES 1 - "Eastern Temperature Series"  
(Emphasis primarily on hypothesis 1; secondarily on 2 and 3; incidentally on 4)

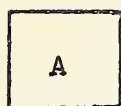
N. J.	Burlington County	Coastal plain	53	--
Tenn.	Morgan County	Ridge & Valley		
		or Appalachian	58	(1) Shortlf. Series 2
Mo.	Dent County	Ozark Plateau	58	--
Miss.	Lafayette County	Older soils	63	--
Ark.	Clark County	Mountain	63	(1) Shortlf. Series 3
Ark.	Ashley County	Coastal plain	63	(1) Shortlf. Series 2 and (2) Clark Co., Ark.
La.	St. Helena Parish	Coastal plain	67	--

SHORTLEAF PINE SERIES 2 - "Western Temperature Series"  
(Emphasis primarily on hypothesis 1; secondarily on 2 and 3; incidentally on 4)

Pa.	Franklin County	(Older hills)	53	--
Ark.	Stone County	Ozark Mountains	58	--
Tenn.	Morgan County	Ridge & Valley		
		or Appalachian	58	(1) Shortlf. Series 1
Okla.	Pushmataha County	Mountain	63	(1) Shortlf. Series 3
Ark.	Ashley County	Coastal plain	63	(1) Shortlf. Series 1, as alternate
Texas	Angelina County	Coastal plain	67	--

SHORTLEAF PINE SERIES 3 - "Botanical Origin and Migration Series"  
(Emphasis primarily on hypothesis 3; secondarily on 2; incidentally on 4)

S. C.	Newberry County	Piedmont	63	--
Ga.	Spalding County	Piedmont	63	--
Ala.	Tallapoosa County	Cambrian or earlier Algon- kian Series	63	--
Miss.	Winston County	(Older soils)	63	--
Ark.	Clark County	Mountain	63	(1) Shortlf. Series 1
Okla	Pushmataha County	Mountain	63	(1) Shortlf. Series 2



← Stand from which seed of Source A was collected.



← Stand from which seed of Source B of same series was collected.

Test plantation in vicinity of Source A

Test plantation in vicinity of Source B

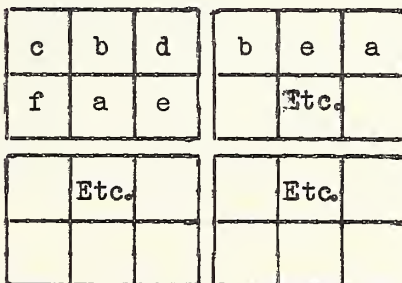
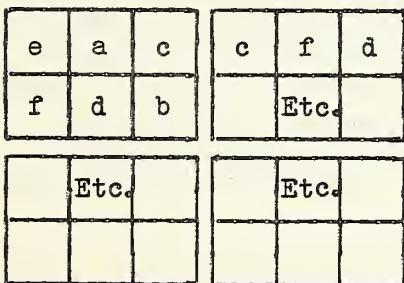
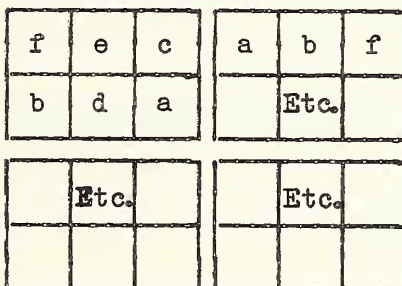
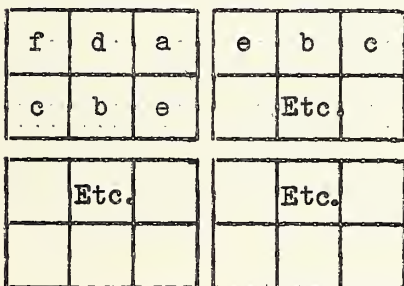
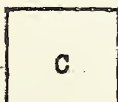
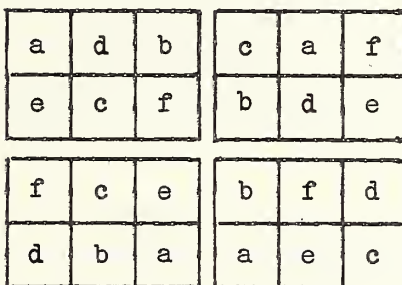
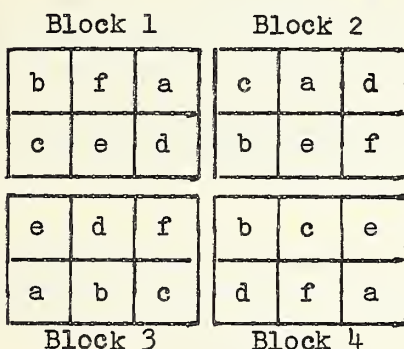


Figure 1.--Schematic layout of seed sources A-F (perhaps each in a different State) of one "series" in the cooperative study of geographic sources of southern pine seed, and of outplantings of the corresponding stock lots a-f in the vicinity of each source in the series.



## SEED

### Quantities of Seed Required

Assuming longleaf seed with the wings on and dewinged seed of slash, loblolly, and shortleaf pine, well cleaned, and with 90 percent or more of full kernels, the quantity of seed of each species required to represent one geographic source in one series, as shown in table 1, will be: longleaf pine, 14.0 pounds; slash pine, 6.2 pounds; loblolly pine, 4.4 pounds; and shortleaf pine, 1.3 pounds. If the seed cuts less than 90 percent sound, correspondingly more seed will be required.

For the sources represented in 2 series as discussed under Experimental Design and noted in table 1, double these quantities will be required. Cooperators from whom such double collections are desired will be so notified through Experiment Station channels.

### Specifications for Collection, Extraction, and Shipment

1. Origin of stand. Seed will be collected from natural stands only. Because of the uncertainty of the geographic sources of seed from which planting stock has been grown in the past, seed from planted trees is useless for the present study.
2. Age of parent trees. Second growth, not less than 20 or more than 40 or at most 60 years old.
3. Site quality. Seed will be collected from stands on sites of average or slightly above average quality for the localities in question.
4. Species. Cooperators will verify the species of each parent tree from which seed is collected, to prevent contamination of seed lots with seed of species other than the one specified for each collection, or of natural hybrids.
5. Number of trees from which to collect. Each seed lot representing one geographic source, whether of standard size (for 1 series) or of double this size (to be divided between 2 series) will be collected from at least 20 trees, with roughly equal numbers of cones from all trees. Without these precautions, the influence of some one genetically peculiar tree might easily obscure the true average growth rate or other characteristics of the local geographic race.
6. Quality of individual tree from which cones are taken. Cones will be taken only from trees of reasonably good stem form, growth rate, and branch habit. They will not be taken from conspicuously crooked, slow-growing, wide-crowned, thick-branched, oddly branched,

or conspicuously diseased trees, although, in the case of slash and loblolly pines, they may be taken from trees lightly to moderately infected with southern fusiform rust, especially if infection in the neighborhood is severe.

7. Date of collection. Cones will be collected only after they have matured fully, as shown by the oil flotation test, and preferably just before they start to open on the tree.

8. Method of collection. Cones will be collected by climbing or from felled trees, as preferred by the cooperator, but in the latter case, only from trees felled after complete maturity of the cones.

9. Extraction. Seed will be extracted promptly after collection, with minimum storage of cones in sacks, bins, or deep piles. Extraction will be at air temperature except where well-proved kilns run by experienced operators are available.

10. Dewinging and cleaning. Immediately after extraction, slash, loblolly, and shortleaf seed will be dewinged by hand or by non-injurious mechanical means; longleaf seed may be similarly dewinged at the option of the cooperator, or left with wings on. Longleaf and loblolly seed will be cleaned, by fanning, to a standard of at least 85 percent with kernels; slash and shortleaf seed will be similarly cleaned to a standard of 90 percent with kernels.

11. Shipment. Immediately after cleaning, and at the latest by December 15, seed will be shipped by parcel post or express prepaid to:

Director  
Southern Forest Experiment Station  
704 Lowich Building  
2026 St. Charles Avenue  
New Orleans 13, Louisiana

The outside wrapper will be plainly marked "Seed for Cooperative Study." Packaging will be secure to prevent loss of seed; when two or more lots are shipped in the same package, each will be enclosed in a separate inside cloth bag, ventilated tin can, or very heavy double paper container to insure against mixture of different lots.

12. Records accompanying seed. Each seed lot will be plainly and securely tagged or labeled, and will be accompanied by a record of:

- a. The collecting agency.
- b. Species.
- c. Location of source (State; county; Section, Township, and Range, or alternatively, distance and direction from nearest town shown on readily available highway maps; and, if

possible, exact latitude, longitude, elevation above sea level, and distance from nearest Weather Station).

- d. Age and general description of parent stand and of actual parent trees.
- e. General description of site.
- f. Date and method of collection.
- g. Period and method of extraction.
- h. Method of dewinging and cleaning.
- i. Any miscellaneous information the collector considers pertinent.

#### Accession, Storage, Testing, and Distribution

Upon receipt at the Southern Forest Experiment Station by the Chairman of the Subcommittee in charge of the study, each lot of seed, whether of standard or double size, will be given a permanent number as described below, and the record accompanying it will be correspondingly numbered, checked, and filed. The seed lot will be examined, recleaned to standard if this proves necessary, sampled for testing, weighed, and placed in cold storage at the W. W. Ashe Nursery, U. S. Forest Service, Brooklyn, Mississippi.

In January, one unstratified 100-seed sample of each longleaf seed lot, and one unstratified and one stratified 100-seed sample of each slash, loblolly, and shortleaf seed lot will be tested for germination on standard peat mats by high-school members of the New Orleans Junior Academy of Sciences.

With the results of these germination tests as guides, the Chairman of the Subcommittee will subdivide each lot of seed in storage into parts of size sufficient to produce at least 1,000 seedlings apiece, place these parts in individual cloth sacks, and number them as described below for distribution to cooperating nurserymen. Tentative weights set up for these parts, prior to accessioning seed lots and making germination tests, are: Longleaf, 1,081 grams apiece; slash, 313 grams; loblolly, 247 grams; and shortleaf, 95 grams. The weights of the parts which will actually be sent will differ somewhat from these values, and will be itemized on forms accompanying the shipments.

During whichever 2-week period from February 15 to April 1 the nurseryman may choose, the Chairman of the Subcommittee will send each cooperating nurseryman his portion of each seed lot assigned to him for sowing. Each nurseryman's consignment will be accompanied by a copy of form SS-26-FM showing the agency undertaking



to sow the seed and produce the stock; the date, place, and manner of shipment; the addressee; the individual making the shipment; and the lot and part numbers of all lots shipped, grouped by species and by series within species. Opposite each lot and part number will be entered the percentage of recleaned seed found to have kernels; the germination percent without stratification; the germination percent (except of longleaf) with stratification; the number of grams of seed being sent to the nurseryman; and suggestions concerning stratification and sowing. At the bottom of the form will be noted the bed space required for all sowing for the cooperative study in the nursery in question. A copy of the completed form will be kept at the Southern Forest Experiment Station, and copies will be sent to the head of the cooperating nurseryman's agency and to the Forest Experiment Station (if other than the Southern Station) negotiating the cooperation.

#### Sample Numbers and Records

Each sample of seed sent to a cooperating nurseryman, and the stock or any portion of the stock raised from it and planted anywhere as part of the cooperative study, will be distinguished by a permanent designation consisting of 3 parts separated by dashes--thus: C-401-6.

The first part of this designation, the capital C (for "Cooperative Study") will be common to all seed and stock lots of all sources in this particular study, to help distinguish them from other seed and stock lots previously numbered by the Southern Forest Experiment Station and other agencies.

The second part of the designation, the lot number, will always be a 3-digit number assigned to the seed lot upon receipt at the Southern Forest Experiment Station. Each lot number will be peculiar to a single seed source and date of collection, and common to all subdivisions of and to all stock grown from the particular seed lot to which it is assigned, regardless of what nurseryman receives the seed and grows the stock or where the stock is planted. In the example given above, C-401 designates shortleaf pine seed collected in Franklin County, Pennsylvania, in the fall of 1951, or stock grown from it.

Lot numbers 101-199 will be reserved for longleaf pine, 201-299 for slash pine, 301-399 for loblolly pine, and 401-499 for shortleaf pine. By this device, the first digit of the lot number will automatically indicate the species of seed or stock.

So far as possible, lot numbers will be assigned to lots of any one species in orderly fashion from north to south, then west, then north again, in geographical order of their sources by States, as follows:

Pennsylvania	Georgia	Texas
New Jersey	Florida	Oklahoma
Maryland	Alabama	Arkansas
Virginia	Mississippi	Missouri
North Carolina	Louisiana	Tennessee
South Carolina		

The exact assignment of lot numbers, including any departures from the foregoing system, will be given in Supplements A and D of this working plan.

Within each species, successive odd lot numbers will be assigned to lots of seed of the 1951 or earlier crops, contributed to the study in 1951 and scheduled for nursery sowing in 1952 to produce stock for outplanting in 1952-53 (possibly in 1953-54 in Pennsylvania, New Jersey, and one locality in Oklahoma). Even lot numbers will be assigned to seed lots received in the fall of 1952 or later, for nursery sowing and outplanting at least one year later than the lots contributed in 1951. By this device any even lot number will, throughout the duration of the cooperative study, distinguish trees younger than those making up the main body of study material. All of the trees in Longleaf Series 3 (see note on this Series in table 1) will be so distinguished. If a plot in any other series has to be left unplanted in 1952-53 because of shortage of stock from 1951 seed, trees from 1952 or later seed used to fill the gap will similarly have even lot numbers.

The third term in the designation, the part number or nursery distribution number, will always be a 1- or 2-digit number. Within a given species the series, nursery distribution numbers will increase in geographical order of States as listed under lot numbers. The nursery distribution numbers will be used in assembling the right parts of the various seed lots for shipment to the appropriate nurseries. In conjunction with lot numbers they will, in addition, designate permanently the nurseries in which particular trees representing particular seed sources were produced. Such knowledge of nursery source may be vital to interpretation of plantation results, because of the frequently great effects of nursery soils and nursery techniques on initial survival and early growth. It will be especially important in any test plantation in which shortage of stock from one nursery has to be made up from a surplus from another. In such cases, the different nursery distribution numbers will simplify recognition of the different stock.

After compilation and checking by the Chairman of the Subcommittee, the collection records for all seed sources represented in the study will be made available to all cooperators in Supplements A and D of this working plan.



## NURSERY STOCK PRODUCTION

### Quantities of Stock Required

Each cooperating nurseryman will sow each seed lot in each series assigned to him to produce 1,000 seedlings, or slightly more than twice the 484 seedlings needed for one test plantation in one locality. Surpluses will be used to offset possible shortages of stock from sowings of the same seed lots in the same or other nurseries, or to establish a second test plantation in the same general locality as the first, as insurance against loss through fire or other causes.

At 30 seedlings per square foot, 36 square feet, or 9 running feet of 4-foot-wide seedbed, will accommodate 1,000 seedlings. The number of running feet of 4-foot-wide bed that each cooperating nurseryman growing seedlings at this density will be called upon to sow will therefore equal 9 times the total number of seed lots assigned to him. It will be stated explicitly in negotiating nursery cooperation, and on form SS-26-FM transmitting the seed.

### General Procedure in Producing Stock

Final decisions concerning most phases of stock production for the study will be made by the individual cooperating nurseryman to fit his particular facilities and schedule of work and the practices found most effective in his particular nursery.

The cooperating nurseryman will determine soil preparation and fertilization, and the date of sowing. Although the Subcommittee may recommend stratification of certain seed lots, final decision as to stratification will be made by the nurseryman in the light of his experience with this treatment, and of the time and equipment available. The nurseryman will decide whether to broadcast or sow in drills 6 inches apart. Although pine-straw seedbed covers are suggested as easier to remove from small plots in which emergence from different seed lots takes place on different dates, the nurseryman will select straw or cloth covers to fit local conditions, including risk of depredations by birds. He will determine the schedule of watering. He will decide whether to weed by hand or with chemical spray, with the proviso, however, that chemicals be used with extreme caution to avoid injury to any sparse or backward seedling stands. He will similarly decide concerning other phases of nursery practice (including the taking of the supplementary data discussed under measurements and records) not covered in the following detailed specifications, and will arrange for any necessary inspection and certification of stock for shipment to the planter.

### Detailed Specifications

1. Soil. To insure good survival and early growth of all lots of stock, and to avoid either obscuring or exaggerating genetic differences among lots during the first years in plantation, stock will be grown on soil which has been shown to produce seedlings of high physiological quality, and is of the maximum uniformity available.
2. Arrangement of lots in bed. In sowing, the seed lots of any one series will be sown one next to another, not interspersed among those of another series or among lots not included in the study. Within any one series, however, lots may be sown in numerical order, or randomized, as the nurseryman prefers.
3. Preserving identity of lots. The stock from each lot of seed will be kept separate from that of adjacent lots by wooden, metal, or tar-paper separators set in the soil of the seedbed and projecting 1 inch above it, to prevent mixing of seed in the event of strong winds or flooding rains before or during germination. Each lot of stock will be marked with a legible, durable label bearing the name of the species and the full designation of the seed lot from which the stock is grown. As insurance against loss of labels, the nurseryman will file at the nursery a verified diagram showing the location within the nursery of the bed in which the seed for the study has been sown and the order of the various lots within the bed, and will send a copy to the Southern Forest Experiment Station.
4. Density of seedling stand. Unless there are strong reasons arising from past experience at the individual nursery, seedlings will be grown from late May or early June at a density of 30 per square foot. If for any reason a standard of fewer than 30 per square foot must be set, the area of seedbed sown with seed of each lot will be increased sufficiently above 36 square feet to accommodate 1,000 seedlings at the lower density.
5. Sowing rate. As a safeguard against discrepancies between observed or estimated laboratory germination and germination in the nursery, and with the notes on form SS-26-FM as guides, seed will be sown to produce stands of somewhat more than 30 seedlings per square foot.
6. Thinning seedling stands. In late May or in June, when danger of damping-off has passed, all seedling stands or portions of stands having more than 30 to 35 seedlings per square foot will be thinned by hand to 30 seedlings per square foot.
7. Protection of seed and stock. Whatever special precautions are locally necessary will be taken to protect seed from birds and

rodents. In any nursery in which it has ever been necessary to spray for control of southern fusiform rust (Cronartium canker), slash, loblolly, and longleaf stock will be sprayed according to the most intensive schedule used in that nursery. Longleaf stock will similarly be sprayed to protect it from brown spot needle blight. With any species of stock suitable poisoned bait, or sprays previously proved effective in the particular nursery, will be used at the first sign of cutworm attack. Sawfly larvae will be controlled with arsenate of lead or DDT. At the first sign of white-grub damage, 20 pounds of 50 percent chlordane powder per acre (1/2 pound per 1,000 square feet) will be applied as a spray. At the first appearance of scale insects, miscible oil emulsion, lime-sulphur, nicotine sulfate, DDT, or HETP will be applied according to manufacturers' directions, at 10-day intervals until the scales are controlled, but only after a preliminary 3-day test on similar or younger stock not included in the study to make sure the treatment does not injure the trees. Other pests will be controlled as they occur. (For details concerning nursery pests and their control, see Volumes 2 and 3 of Southern Forest Experiment Station Occasional Paper 122.)

#### Measurements and Records

In September each cooperating nurseryman will determine the number of potentially plantable seedlings in each lot of stock he is growing for the study, in multiples of 125 (to facilitate preparation of shipping lists), and report the numbers promptly to the Chairman of the Subcommittee, on forms to be provided by the Southern Forest Experiment Station. (Definitions of "plantable seedlings" will be included in the forms.)

Except for these counts, which are essential for scheduling the shipping and planting of the stock, no records or measurements will be required except in a few cases to be dealt with by individual correspondence. Nurserymen who can supply the following supplementary data concerning each lot of stock in one or more series will, however, greatly strengthen and enrich the study:

- a. Date of general formation of secondary needles.
- b. Date of general formation of winter buds.
- c. Average height of tops (general level in bed, or, better, average of 20 randomly selected seedlings) at time first lot in series forms winter buds as in b.
- d. Average height of tops (taken as in c) immediately before lifting.
- e. Notes (if possible with sketches or photographs) on any clear-cut differences in root habits of longleaf pine seedlings from different seed sources.



- f. Notes on any miscellaneous points of interest, such as differences in dates or shades of color change with coming of cold weather.

### Lifting, Grading, Culling, Packing, and Shipping

The four things of paramount importance concerning lifting and shipping are that: (a) Stock will be lifted and shipped at the date specified by the planter, notice of which will be transmitted to the nurseryman well in advance, either directly by the planter or through the Subcommittee or other channels; (b) all stock will be shipped in 125-seedling units, each securely tied to prevent mixing or loss, and each marked with a weatherproof wooden label supplied by the Subcommittee; (c) stock will be kept moist at all times by scrupulous care in handling, packing, shipment, and heeling-in; and (d) unless other specific arrangements have been made, the nurseryman will provide all inspection certificates necessary for shipment and will prepay the shipment.

Further detailed specifications for lifting, etc., are:

1. Care in preserving root systems. Whether seedlings are undercut by machine or lifted entirely by hand, extreme care will be used to avoid cutting roots shorter than 8 inches, and to separate seedlings from soil without loss of lateral roots.
2. Discarding misplaced stock. Seedlings displaced past the boundaries of their respective seedbed plots during undercutting or lifting, or otherwise misplaced during grading or packing, so that there is any doubt as to the lot to which they belong, will be discarded.
3. Grading and culling. Because of anticipated variations in seedling size with origin of seed, seedlings will be shipped and planted bed-run instead of being graded to size in the usual manner. Mechanically injured seedlings, seedlings with root systems less than 5 inches long, seedlings with tops less than 3 inches long, (in the case of longleaf, with maximum needle length less than 6 inches), and all seedlings with visible fusiform rust cankers, living scale insects, or 20 percent of the foliage in brown-spot lesions, will, however, be culled. In longleaf pine lots, all Sonderegger pine seedlings will be culled.
4. Root pruning. All roots will be pruned to  $7\frac{1}{2}$  to 8 inches.
5. Packing. Stock will be tied with soft cord to hold seedlings firmly but without injury. It will be packed in moist sphagnum moss, not in sawdust, granulated peat, or other material more finely divided or less moisture-retentive than sphagnum. It will be wrapped in reinforced waterproof paper or equivalent material in a way to keep roots moist and tops adequately but not excessively aerated, and to prevent loss or injury during shipment.

6. Grouping and identification of seedlings. The seedlings of any one stock lot in any given series in a particular nursery may go to one planter only, or to two or more planters, as shown in the shipping directions. In any event, however, they will be shipped in 125-seedling units, each correctly tagged with a weatherproof wooden label. (With most lots of stock, it probably will be necessary to combine 2 or 3 smaller bundles, with moss between them, to make a 125-seedling unit that will stand shipment safely.) These 125-seedling units will be packed in small bales each containing the six, seven, eight, or nine units allocated to one block of the test plantation for the series in question. The procedure in putting up these units and bales will be as follows:

- a. To avoid risk of mixing or mislabeling stock, all the 125-seedling units scheduled by the shipping directions to come from a given lot of stock in a given seedbed plot will be lifted, counted out, tied, labeled, and either covered with wet moss and burlap or else temporarily heeled-in, before any seedlings in adjacent or nearby seedbed plots are freed from the soil. As each 125-seedling unit is labeled, its number will be checked off on the shipping directions sheet for the series to which it belongs.
- b. When all six, seven, eight, or nine lots of a given series have been lifted, tied, and labeled in this way to the extent called for by the shipping directions, the six to nine 125-seedling units representing the six to nine stock lots for Block 1 of a given planter's test plantation will be assembled and packed in a bale which can be delivered to that particular block. (The checked shipping directions and the tags supplied by the Subcommittee will provide the information necessary for this assembling.) The process will be repeated to make separate bales for Blocks 2, 3, and 4 of the same test plantation. If supplementary 125-seedling units of stock from the same series go to one or more additional planters, they will be assembled and baled for them in like manner. Depending on the size of the seedlings and the number of lots to be planted per block, the four bales of stock for a single test plantation may be bound together for shipment, or shipped separately, whichever is more convenient and will insure arrival in the better condition.
- c. The utmost care will be taken throughout, first, to see that the 125-seedling units are correctly grouped according to the information on the labels, and second, that the roots are kept moist during packing and will remain moist in transit.

## PLANTING

### Locations, Sites, Areas, and Planting Stock Required.

Facilities for cooperation permitting, the outplanting in the vicinity of each seed source represented in the study will be within 5 or 10 miles of the stand or stands of parent trees constituting the source, and within 200 to 500 feet of the same elevation above sea level. A location more than 50 miles from the source in question will in no case be chosen without first exhausting the possibilities of finding a suitable location within 50 miles, and even then only after clearing the choice with the Subcommittee.

Each outplanting will be established on the most uniform site of average or somewhat better than average quality for the locality that is available to the cooperating planter in a place where he can protect the plots effectively. With the exception of the sand-hill sites for Longleaf Series 2 in North Carolina, South Carolina, and Florida, where deep sands are part of the test, very adverse sites, even though uniform, will be avoided, lest mortality be excessive. Open sites will be preferred to sites from which brush must be eliminated to insure uniformity and good survival and growth. Land not previously cultivated will be preferred to old fields, except in localities where forest planting is predominantly on old fields. Actively eroding sites will in all cases be avoided.

The net area required for outplanting each series of longleaf, slash, or shortleaf pine in the vicinity of any given seed source represented in the series will ordinarily be 2.4 acres, and in no case more than 2.8 acres. The ordinary and maximum areas for each loblolly series will be 3.2 and 3.6 acres. The number of series to be outplanted and the exact areas required will be itemized and totaled for each planting locality in the study, both in Supplements C and D of this working plan and in correspondence with individual cooperating planters.

Each test plantation of a given series will require 484 seedlings of each lot of stock represented in the series, 121 in each of the 4 blocks making up the test plantation. As a margin of safety, 500 seedlings of each lot will be shipped to the planter, in 4 units of 125 seedlings each (1 unit for each block) as noted in the preceding section.

### Arrangement of Planted Trees in Plots and Blocks

Each individual plot in the test plantations will be square, 66 feet on a side, and planted with 11 rows of 11 trees each, spaced 6 by 6 feet, with the outermost rows and trees 3 feet inside the plot boundary. All 121 trees within any one plot will be from the same seed source and produced in the same nursery.



Each test plantation for a given series will consist of 4 blocks containing equal numbers of such plots. The number of plots per block will be 6, 7, 8, or 9, depending upon whether 6, 7, 8, or 9 geographic sources of seed are represented in the series. Unless limited size and odd shape of the planting space requires some other arrangement, the 6, 7, 8, or 9 plots within any given block will be arranged as shown in figure 1 and in figure 2A, 2B, and 2C, respectively.

Plots and blocks will be laid out so that any unavoidable differences in soil and cover conditions, such as are likely to exist on even the most uniform sites available, occur between one block and the next, rather than among the 6 to 9 plots in any one block. The importance of uniformity within the individual block cannot be too strongly emphasized.

Subject to the specifications in the preceding paragraph, each cooperating planter will decide whether he can best protect any one test plantation by laying out the 4 blocks immediately adjacent one to another, separating them by plowed or harrowed fire breaks, or laying them out 100 yards to 1 mile apart. On cut-over longleaf pine land, for example, where fires run fast and hogs are hard to exclude, 4 blocks on 4 successive ridges may give adequately similar soil conditions from block to block and at the same time involve less risk of total loss than would the use of 4 immediately adjacent blocks.

In the test plantation for any one series in one locality, stock grown from each seed source represented in the series will be planted on one plot, but on only one plot, in each and every block in the plantation. This is illustrated by stock of sources "a", "b", "c", "d", "e", and "f" in the diagram for the test plantation in the vicinity of Source A, in figure 1.

The 6 to 9 sources in each block will be assigned at random to the plots within that block. Form diagrams patterned after figure 1 or figure 2A, 2B, or 2C and showing the randomization of the specific lots of stock assigned to each cooperating planter will be sent to the planter, in duplicate, by the Chairman of the Subcommittee, well in advance of planting. When planting has been completed, the planter will return one copy to the Chairman, endorsed to show that the randomization has been followed (or annotated to show any necessary corrections or changes), and marked with compass directions to show orientation of the blocks. These returned forms will constitute the backbone of the record of establishment of test plantations.

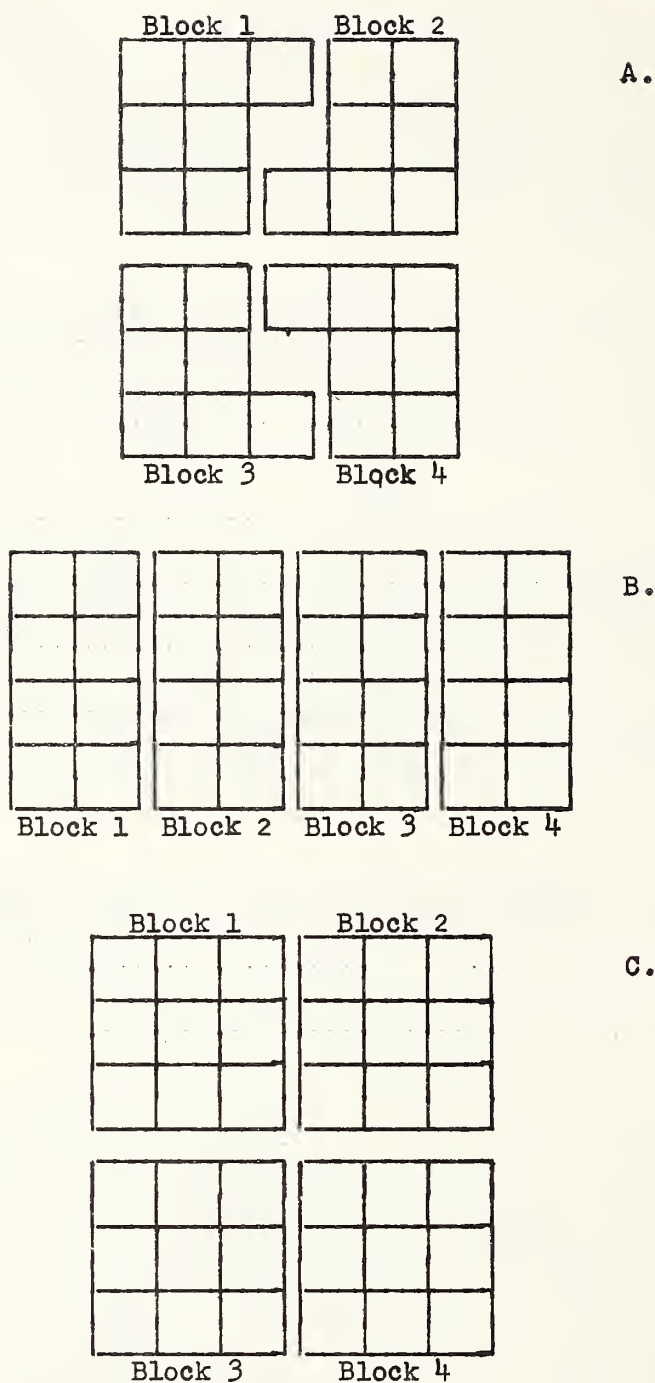


Figure 2.--Arrangements of plots and blocks for: A, test plantation including stock from seed from 7 different geographic sources; B, from 8 sources; C, from 9 sources. Random assignment of individual sources to plots within each block will be made for the planter by the Subcommittee in advance of planting.



## Planting Specifications; Establishment Records

1. Date of planting. Each cooperating planter will choose whatever date of planting assures the best chance of good initial survival in his particular locality. The nurseryman producing the stock for the planter in question will ship it (on direct request from the planter or through the coordination of the Chairman of the Subcommittee) to meet this date.
2. Site preparation. Each cooperating planter will apply whatever special site preparation, if any, gives best results on his sites in his locality, except that, to avoid increasing the rate of infection by fusiform rust, slash and loblolly pine planting sites will in no case be cultivated or fertilized.
3. Care of stock on arrival. Stock will be inspected on arrival and its condition recorded, with detailed notes on anything that may adversely affect part or all of the seedlings. Stock received with packing in poor condition, and stock to be held for more than 48 hours before planting, will be unpacked, and the 125-seedling units heeled-in without being untied, but with special care to cover roots properly.
4. Method of planting. Because of the small size of the plots and the necessity of keeping lots of stock separate by plots and of preserving alignment, all planting will be by hand instead of by machine. Whatever planting tool is preferred locally will be used. Special care will be taken to set the trees at the same depth as that in which they grew in the nursery, to get the roots straight in the planting slits, and to close the slits firmly.
5. Spacing. By means of steel tapes or suitably marked wires, chains, or well-stretched strings, trees will be spaced accurately, 6 feet apart in both directions, in 11 rows of 11 trees each on each plot, with the outermost trees all around 3 feet inside the plot boundary.
6. Marking of blocks and plots. In advance of planting, all block and plot corners will be marked with tall, substantial metal, treated wooden, or durable heartwood posts. Block corners will be labeled (by scribing, or with durable painted or metal tags) to show species, series, and block number. Plot corners will bear stock lot designations in full, so oriented or indicated on maps as to show unmistakably the plots to which they apply. Desirable additions are: (a) small durable stakes marking  $42 \times 42$  foot square (7 rows of 7 trees each) in the center of each plot, to which all remeasurements of the stock on that plot will be confined; (b) a legible, durable sign on each such square, giving stock lot designation and also State and county or district of seed origin (see Supplements A and D); and (c) an over-all sign on each block or group of blocks in a series, giving species, series, and name of cooperating planter under the title "Cooperative Study of Geographic Sources of Southern Pine Seed."

7. Record of establishment. This will consist, for all outplantings in a given locality by a given cooperator, of: (a) a description of the planting locality--State, county, distance and direction from nearest town shown on road maps, land ownership, soils and cover, and, if possible, latitude, longitude, elevation above sea level, and nearest Weather Station; (b) an over-all map showing the locations of all blocks in all series in the locality, tied in to Public Land Survey corners or equally permanent and identifiable landmarks; (c) the confirmed or corrected copy (previously referred to) of the randomization scheme of each block; (d) a record of the dates of receipt and of planting of all stock, its condition on arrival; its manner of storage, and its condition when planted; (e) notes on weather and other influences, during and shortly after planting, which may affect initial survival; and (f) (for the 49 "measurement trees" only, in inner central square of each plot) the height, of each seedling (except longleaf), in inches and tenths, from ground to base of bud (or to base of topmost living needles if no bud is present) immediately after planting. Forms for recording the foregoing will be sent to individual planters from the Southern Forest Experiment Station. One copy of the complete establishment record, including maps, will be retained by the planter, and one will be sent to the Chairman of the Subcommittee at the Southern Station. The chairman will prepare a verified, harmonized compilation of the records for all plantations included in the study, to be filed in duplicate at the Southern and Southeastern Stations.

#### Protection, Thinning, and Pruning

Some forms of injury may help differentiate geographic races. Others may nullify the study by destroying the test plantations. Special protection will in general be withheld in the case of injuries of the former type, and applied in the case of the latter. (For details of control, see Southern Forest Experiment Station Occasional Paper 122, Vol. 3.) More specifically:

1. Wildfire, hogs, sheep, and goats. These will be strictly excluded. Individual planters will choose the most effective means in their particular localities, including special firebreaks and fences where necessary.

2. Cottontail rabbits. No special measures will be taken against rabbits except by planters producing their own stock for the study and planting it in localities where heavy rabbit damage ordinarily necessitates use of rabbit repellents.

3. Early drought. Except by prior arrangement with the Subcommittee, Dowax or similar foliage coatings will not be used.

4. Pocket gophers (Geomys). These will be controlled promptly, by traps or poisoned bait, whenever discovered in or near test plantations.



5. Texas leaf-cutting ants. These will be controlled promptly, with methyl bromide or other well proved insecticide, whenever discovered in or near test plantations.
6. Nantucket tip moths. These will be left uncontrolled except in localities (Oklahoma ?) in which cooperating planters have found spraying with DDT or other substances essential to survival.
7. Colaspis beetles. Whenever these appear in or near test plantations, they will be controlled promptly with benzene hexachloride, chlordane, DDT, or arsenate of lead.
8. Sawfly larvae. These will be controlled promptly, the same as Colaspis beetles.
9. Brown spot needle blight. Since longleaf pine cannot ordinarily be planted successfully without controlling this disease, brown spot will be controlled in longleaf pine test plantations in all localities in which more than a trace of brown spot occurs. Control will be by spraying individual seedlings (including those in the border strips as well as the "measurement trees" in the centers of the plots) with Bordeaux mixture or other proved fungicide, plus a good adhesive, each May and November until the great majority of trees are at least 18 to 24 inches tall. (Because of the small areas of the test plantations, and the danger of losing trees through anything short of perfectly timed and executed burning, prescribed burning will be used instead of spraying only by special arrangement with the Subcommittee.)
10. Southern fusiform rust (Cronartium fusiforme). Since differences in susceptibility to this disease among different races of slash and of loblolly pine are of outstanding interest in the present study, no control will be applied in the plantation phase, except that (a) slash and loblolly pine test plantations will in no case be cultivated or fertilized after or at the time of planting; and (b) prescribed burning will not be resorted to for hazard reduction in slash pine plantations before the trees average 15 feet high. Infected twigs will in no case be pruned to keep cankers from reaching the trunks.
11. Cronartium cerebrum on shortleaf pine. This will not be controlled.

Thinning. Plots, including the border strips, will be thinned according to uniform specifications designed to (a) prevent marked slowing down of diameter growth in any plots; (b) permit direct comparison of total wood production--live stumpage plus yields from thinnings--among all plots in the study; and (c) avoid reduction of yield through waste of growing space by excessive artificial opening up of the stand. Details of thinning will be specified in a supplement to this working plan, prepared in the light of the 10-year reexamination.

Pruning. Test plantations will not be pruned at any time, as differences in branch habit, rate of self pruning, and the like may throw important light on differences in geographic races.

## PLANTATION INSPECTIONS AND REEXAMINATIONS

### General Inspections

Between detailed reexaminations, each cooperating planter will keep his test plantations under close enough observation to catch, at the start, any injuries by animals, insects, or disease, and will report to the Chairman of the Subcommittee any appreciable injuries from these or other causes, <sup>and</sup> any steps taken to control them. In more northerly localities, special attention will be given, in the first general inspection in the spring, to evidences of winter killing or frost injury; if such injury occurs, a quantitative record will be made of any differences observed among stock lots.

### Detailed Reexaminations

Routine reexaminations involving detailed counts and measurements will be made by individual cooperators establishing or maintaining test plantations. The Subcommittee will schedule these with the cooperators and will supply forms, and will also arrange for any special reexaminations--by its own members, by forest pathologists or other specialists, or by the cooperating planters--as need arises. The Chairman of the Subcommittee will maintain a list of reexaminations completed and to come.

The minimum schedule of routine reexaminations will be: (a) June or late May of the first growing season in plantation; (b) end of the first growing season in plantation; (c) end of the third growing season in plantation; and (d) end of the fifth growing season in plantation and end of every fifth growing season thereafter. For their own information some cooperators will undoubtedly re-examine their test plantations more frequently, especially during the first ten years. Certain special examinations during one or another growing season, to learn differences in dates of bud opening and bud formation, will be requested of selected cooperators.

### Data to Record

Unless special arrangements are made to the contrary, routine reexamination of each plot will be confined to the 49 "measurement trees" in the 42 by 42 foot central square in each plot. The following data will be recorded:

- a. Location, cooperating planter, species, series, block number, stock lot designation in full, State and county (or district) of seed origin, date of reexamination, and name and title of observer.

- b. The condition of each tree, whether living, dead, or missing.
- c. The height of each living tree, in feet and tenths, from ground to base of winter bud or of highest living portion of stem.  
(May be omitted in reexamination of all species during May or June of first growing season, and in reexamination of longleaf at end of first growing season.)
- d. In longleaf only, and only at the end of the first growing season, the condition of the bud--whether elongated, spherical, or flat ("pincushion bud").
- e. In the June (late May) reexamination of the first growing season only, rabbit damage, except to longleaf. (Necessary to distinguish any preferences of rabbits for one over another lot of stock, and to interpret later records of survival and height.)
- f. The breast high diameter, in inches and tenths, of each tree more than  $4\frac{1}{2}$  feet high.
- g. In all reexaminations up to the time of the first thinning, southern fusiform rust infection of each longleaf, slash, and loblolly pine, tallied separately as on branches only, as on trunks only, or as on both.
- h. Infection of shortleaf pine by Cronartium cerebrum, tallied as in g above.
- i. For longleaf only, an estimate of the general extent of brown-spot infection on each plot, expressed as range of, and average, percentage of current year's foliage involved in brown-spot lesions or killing. (This will serve as a check on the need for spraying, the effectiveness of spraying, and the possible effect of infection on survival and growth.)
- j. Where it occurs, ice damage on each tree, by classes or by degrees of severity.
- k. In as precisely quantitative terms as possible (individual tree tally, with or without severity classes), any other form of injury that may vary from lot to lot of stock and hence help to distinguish geographic races, or that appears likely to reduce appreciably the survival or growth of any or all lots. These will be followed up by notes on any control measures applied and control attained.

Wherever possible, the following supplementary data will be taken on 25 randomly selected trees on each plot (or on all trees if fewer than 25 survive), in one or more growing seasons before the trees average 5 feet in height:



Either (a) the numbers of trees which have, and also the numbers of trees which do not have, one or more needle tips exposed by emergence from the needle sheaths in the terminal buds, at weekly intervals from the time the first needles appear on the earliest starting stock until all the trees in the latest starting stock have needles exposed; or (b) the length, in inches and tenths, of the longest needle exposed beyond the needle sheath on the terminal bud, on the date when the latest-starting stock first exposes its needles.

(c) The numbers of trees which have, and also the numbers of trees which do not have, distinct winter buds, at weekly intervals from the time the earliest lot of stock forms winter buds until all lots have formed winter buds. Buds tallied during the first few counts will be touched with paint, as a check against their elongating and forming new internodes before true winter buds are formed.

(d) The numbers of internodes formed by each tree in the current year.

Originals or verified transcripts of all data will be sent to the Chairman of the Subcommittee promptly after completion of each re-examination.

#### ANALYSES OF RESULTS

As the first step in evaluating results, the Subcommittee will test the significance of differences in survival, height, infection, etc., among the various seed sources represented in each series in each planting locality. This will be done rigorously by analysis of variance, using the arc sin  $\sqrt{\text{percentage}}$  transformation for variables expressed in percents. The usual breakdowns, assuming four blocks and depending on number of geographic sources in the series, will be:

Source of variation	Degrees of freedom when number of geographic sources in series is:-			
	6	7	8	9
Block	3	3	3	3
Geographic source of seed	5	6	7	8
Error	15	18	21	24
Total	23	27	31	35

Further steps in evaluation will be:

- a. Tabulation of consistent trends (as of variation of results with temperature, rainfall, or latitude of seed source, or with

distance of source from planting site) and of reversals (as failure of southern seed on northern sites and northern seed on southern sites) within each series, and among different series of the same species.

- b. Evaluation of the hypotheses tested by means of the different series of each species, to see which best explains the origin or distribution of geographic races within that species. (As a starting point, it may be assumed that the series exhibiting the greatest variance in one or several planting localities, and especially where two series are planted side by side, illustrates the strongest hypothesis.)
- c. Checking of results under a and b in one species against corresponding results in one or more other species, to see whether different species show a common pattern.
- d. An examination of results from all species, separately and together, for evidence of invasion of distinct territories by different genetic entities migrating from separate ancient land masses, more or less independently of present temperature or rainfall zones. (A sharp north and south boundary between races of loblolly pine highly susceptible to and highly resistant to southern fusiform rust might constitute such evidence.)
- e. A joint evaluation of results in both loblolly pine series, and in all three longleaf pine series and shortleaf pine series, made by expressing average heights, etc., of all lots in any two series as percentages of the average heights, etc., of lots common to both series.
- f. Mapping of tentative racial distributions and seed collection zones from results of the foregoing steps.

In making these analyses, initial survivals will be accepted cautiously; in particular, care will be taken not to credit racial variation with differences more logically attributable to nursery origin of stock, delayed germination, injury to stock in transit, or random accident after planting.

#### SALVAGE IN THE EVENT OF MISHAP

In an enterprise as extensive and complex as the present study, some obstacles and limitations exist from the start, and complete freedom from later mishaps is unlikely.

Limitations already encountered and mishaps anticipated include non-production of seed at sources it is desired to include, collection of seed at other than specified locations, collection of

insufficient seed, germination failure in the nursery, production of fewer than 500 trees (absolute minimum, 484 trees) from a given lot of seed in the nursery, injury to stock of a given lot in shipment or storage, and loss of planted trees from fire, hogs, and the like.

The over-all design of the study and the contribution of seed from several alternative sources provide the first recourse in getting the desired information from the study despite such limitations and mishaps. Where seed has proved unavailable, non-viable, or wholly inadequate in amount, series have been redesigned or alternative sources have been used. In one longleaf series, collection, sowing, and planting have been deferred till a later year, and a few gaps in other series will similarly be filled at a later date. Shortages in individual lots of stock will be made good, so far as possible, with surplus stock grown from seed of the same original lot in the same or other nurseries. In the event shortages cannot be made good, the lots will be omitted from one or two of the four blocks of the test plantation involved. Where adequate surpluses are available, complete duplicate outplantings will be made by additional cooperators in the vicinities of various seed sources, as insurance against wholesale losses of test plantations, particularly by fire. Details of these adjustments will be set forth in Supplements A through D of this working plan, and in individual correspondence with the cooperating nurserymen and planters concerned.

The ultimate insurance against mishaps is that each test plantation of one series is rigorously analyzable as a unit, independently of any other test plantation, and capable of yielding substantial information concerning geographic races and acceptable seed sources of the species it represents. Moreover, while sensitivity will be reduced if one or two blocks of the test plantation are incomplete through shortage of stock or through injury after planting, even two intact blocks will make possible a rigorous test of the significance of differences among the lots of stock planted.

In the event of any actual or suspected mishap or derangement in the study, the first and most important move will be to preserve alive and label unmistakably the seed or seedlings involved, record fully and exactly what has happened and any attending circumstances that may clarify the situation, and forward the record to the Chairman of the Subcommittee at the Southern Forest Experiment Station. The Subcommittee will then decide the course of action on the evidence available.

Three other general rules are:

1. Except by specific prior arrangement with the Subcommittee, stock of a given lot grown in a particular nursery for outplanting in a particular locality, and surpluses of other



lots of stock of the same seed source grown in the same or other nurseries and shipped to make good any shortages in the first lot, will be shipped only in bundles of 125 (absolute minimum, 121) seedlings. The purpose of this rule is to insure that all trees on a given plot are of the same nursery origin as well as of the same seed source.

2. In the event that 49 or more seedlings, but fewer than 121, are available for a given plot, the interior square of "measurement trees" will be planted at 6 by 6 foot spacing, and the extra trees used for as close and uniform a single or double-row border strip as possible around all four sides of this interior square.
3. In the event that any given plot in a slash or loblolly test plantation is left unplanted the first year, for lack of stock, and is filled later by stock grown from seed subsequently collected at the designated source, supplementary plots will be planted at the same later date with stock grown from subsequent collections at one or more other sources in the same series. This precaution is necessary to measure the highly variable effects of age of seedlings and of weather in different years upon incidence of southern fusiform rust. Details of such supplementary plantings will be arranged by the Chairman of the Subcommittee in individual correspondence with the cooperating collectors, nurserymen, and planters concerned.

## APPENDIX

### Details of Hypotheses Concerning the Origin and Distribution of Geographic Races of Southern Pines

Four conflicting hypotheses concerning the origin and distribution of geographic races of southern pines are stated in very general terms on page 5 of this working plan. The following detailed discussion of these four hypotheses, with a note concerning a fifth, should make clear the fundamental relationship of these hypotheses to the design of the present study and the interpretation of results.

Hypothesis 1 is that different geographic races of any given species have evolved in response to, or at least are most closely associated with, differences in temperature. More specific statements of this hypothesis assume (a) average annual temperature; (b) average temperature during the "growing season" (variously defined); (c) average maximum temperature; (d) extreme maximum temperature; (e) average minimum temperature; (f) extreme minimum temperature; (g) length of frost-free period; or (h) some combination of the foregoing.

Since (b) through (h) are all strongly correlated with (a)--average annual temperature--and are far less easily utilized in the preliminary stages of a study like the present one, average annual temperature will be used as a guide in devising tests of temperature relations in the present study. This by no means rules out interpretation of the effects of (b) through (h) in analyzing results.

A frequently stated or implied corollary of hypothesis 1 is that if seed from one place in a given temperature zone proves well adapted to a given planting location, seed from anywhere else in the same temperature zone will be equally well adapted to that location, or at least fairly well adapted, and better adapted than seed from any other temperature zone.

Two frequently suggested elaborations of hypothesis 1 are mutually contradictory. One is that (in the Northern Hemisphere) seed should be moved north rather than south, to avoid loss of growth through incomplete utilization of the growing season. The other is that seed should be moved south rather than north, to avoid mortality or loss of growth as a result of insufficient cold-hardiness.

Hypothesis 2 is that distinct and important geographic races have evolved in response to, or at least are most closely associated with differences in rainfall, major soil type, local populations of parasitic fungi, and the like, even within zones of similar temperature conditions; in extreme form, that such geographic races extend across temperature zones and that the differences between them exceed the differences associated with temperatures. If this hypothesis be sound, it invalidates the corollary under hypothesis 1.

Hypothesis 3 is that the origin and territorial extent of geographic races within a species bear little or no relation to differences in climatic conditions, soils, or other influences now existing within the species range, but have resulted primarily from evolution on different isolated land masses projecting above former more extensive seas, and from the routes of migration followed as the pines invaded areas more recently elevated above sea level. The Ozark and Ouachita Mountain area, the southern end of the Appalachian Mountains and adjacent Piedmont, and the Apalachicola Bluffs in Florida, are ancient land masses on which different geographic races of pines may have evolved. Migration may have been affected, in ways not yet understood, by the successive shifts of the Mississippi River, with its accompanying barrier of wide flood plains, from the Neches to the Sabine to the Atchafalaya to its present course.

Hypothesis 4, in conflict with all three of those just stated, is implied in the formerly common assumption that there are no geographic races among the southern pines, and that, for any given planting location, seed may be obtained indiscriminately from any part of the species range without significantly affecting the survival or growth of the planted trees. This hypothesis has been thoroughly disproved in the case of loblolly pine and fairly well disproved in the case of shortleaf, and is at least suspect in the case of longleaf pine. There is as yet, however, little or no evidence against it in the case of slash pine native to the continental United States--the distinct variety called "South Florida slash pine" excepted.

A fifth hypothesis, suggested by a few minor articles on American pines and by an as yet unpublished thesis by Perry on a western species of poplar, is that geographic races of southern pines may be associated with differences in photoperiodism or day-length. In the relatively low latitudes and generally low altitudes of the southern pine region, however, differences in day length can only with difficulty be studied separately from differences in average annual temperature, with which they are strongly correlated through joint correlation with latitudes. Day-length may have to be considered in interpreting tests of hypothesis 1, concerning the relation of geographic races of southern pines to temperature zones, but will not be subjected to independent test in the present study.







